International Journal of Pharmaceutics 566 (2019) 291–298

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Development of PLGA microparticles with high immunoglobulin G-loaded levels and sustained-release properties obtained by spray-drying a water-in-oil emulsion

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ARTICLE INFO

Keywords:
Spray-drying
Poly(lactide-co-glycolide) acid (PLGA)
Sustained-release
Antibody
Water-in-oil emulsion
Microparticles

ABSTRACT

In this study, the possibility of producing highly antibody-loaded microparticles with sustained-release properties was evaluated. Polyclonal immunoglobulin G (IgG) was used as a model of antibody and its encapsulation into poly(lactide-co-glycolide) acid (PLGA) microparticles was performed by spray-drying a water-in-oil (w/o) emulsion. It was demonstrated that the use of the Resomer® RG505 PLGA allowed an IgG loading of 20% w/w with an encapsulation efficiency higher than 85%. The produced microparticles were characterized by a mean diameter lower than 10 µm. The burst effect was shown to reach a maximal value of 40%. IgG stability after encapsulation was also assessed. The use of this single PLGA provided a lag time of 3 months which dramatically slowed down the release rate after the initial release of the encapsulated IgG. Using blends of PLGA characterized by different inherent viscosities allowed decreasing the lag time and modulating the dissolution profile of the IgG from the spray-dried microparticles. Therefore, spray-drying a water-in-oil emulsion appeared to be a promising strategy to produce highly antibody-loaded microparticles characterized by sustained-release properties.

1. Introduction

Since the approval of recombinant human insulin in 1982, a growing interest for the use of biotherapeutics was observed due to their obvious advantages compared to small chemical entities. Indeed, being physiologically produced by the body, such macromolecules are well tolerated and highly specific, which reduces the appearance of adverse effects (Leader et al., 2008). Among biotherapeutics, antibody-based products constitute the largest and fastest growing class with a global sales revenue of nearly $75 billion in 2013 and over 300 antibody candidates in development (Ecker et al., 2015). Nowadays, they are recognized as targeted therapies for malignancies, transplant rejection and both autoimmune and infectious diseases (Hansel et al., 2010).

Due to the relative low bioavailability observed when biotherapeutics are administered by noninvasive routes (e.g. oral route), such biomolecules are commonly parenterally delivered (Mitragotri et al., 2014). However, using conventional parenteral dosage forms, frequent injections are required to maintain the drug concentration into the therapeutic window due to their reduced serum half-life, especially for fragments of antibodies. This may reduce the compliance of patients (Vaishya et al., 2015) as well as induce peak to trough fluctuations in blood levels due to multiple dosing. To avoid these drawbacks, the development of sustained-release formulations has been intensified over the past few years (Schwendeman et al., 2014; Vaishya et al., 2015). Nevertheless, controlled-release delivery systems for proteins must meet several specific criteria. For instance, they should be characterized by high drug loadings (DL) to allow the administration of therapeutic doses (Ye et al., 2000) as well as by a continuous and sustained-release profile over time. These formulations should also maintain the physicochemical stability of the proteins through both production and delivery to avoid immunogenicity issues (Jiskoot et al., 2012).

Abbreviations: BCA, bicinchoninic acid; DCM, dichloromethane; DL, drug loading; DSC, differential scanning calorimetry; EE, encapsulation efficiency; EtAc, ethyl acetate; ExE, extraction efficiency; T_g, glass transition temperature; IgG, immunoglobulin G; PBS, phosphate-buffered solution; PLGA, poly(lactide-co-glycolide) acid; SE-HPLC, size exclusion high-performance liquid chromatography; SEM, scanning electron microscopy; s/o/w, solid-in-oil-in-water; w/o, water-in-oil; w/o/w, water-in-oil-in-water

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https://doi.org/10.1016/j.ijpharm.2019.05.070
Received 28 February 2019; Received in revised form 24 May 2019; Accepted 27 May 2019
Available online 28 May 2019
0378-5173/

Biodegradable microparticles have been widely investigated for the controlled delivery of biotherapeutics (Sinha and Trehan, 2003). These systems are based on the encapsulation of the protein into a carrier which allows its protection against degradation as well as its controlled release over time. Moreover, the use of biodegradable polymers (e.g. poly(lactide-co-glycolide) acid (PLGA)) allows avoiding both surgical removal and toxicity related to non-biodegradable microparticles. In order to be injected through needles with acceptable diameters, such microparticles must be characterized by an appropriate particle size distribution and, more particularly, a mean diameter lower than 125 µm (Kissel et al., 2005).

PLGA polymers have been widely used as carriers for protein-loaded biodegradable microparticles due to their biocompatibility and their regulatory approval for parenteral human use by both Food and Drug Administration and European Medicines Agency (Blanco and Alonso, 1998; Cho and Sah, 2005; De Alteriis et al., 2015). Conventional protein-loaded PLGA microparticles exhibit typical triphasic *in vitro* release profiles comprising (i) an initial burst, (ii) a lag phase and (iii) a release step (Diwan and Park, 2001; Igartua et al., 1998; White et al., 2013). Neither a burst release that cannot be controlled, nor the presence of a lag phase are desirable as it can be associated with adverse effects linked to peak serum exposure (Mitragotri et al., 2014) and prevents the continuous delivery of the protein, respectively. Nevertheless, by modifying the physicochemical properties of PLGA, such as the copolymer ratio or the molecular weight, it is possible to modulate both burst effect and duration of the lag phase (Yeo and Park, 2004). However, it was frequently observed that the use of a single PLGA cannot control meanwhile the burst release and the lag phase duration (Zolnik et al., 2006). Therefore, the use of blends seems to be an interesting alternative to produce microspheres with more suitable release profiles (Wang et al., 2014).

Encapsulation of macromolecules is usually performed using double emulsion techniques such as water-in-oil-in-water (w/o/w) or solid-in-oil-in-water (s/o/w) emulsions, followed by solvent evaporation and/or extraction (Blanco and Alonso, 1998; Marquette et al., 2014a). However, non-negligible amount of protein may be lost in the external aqueous phase, leading to a significant decrease of the DL. For instance, Wang et al. obtained a DL of 6.7% and 3.3% w/w from the w/o/w process and the s/o/w process, respectively (Wang et al., 2004). Similarly, Marquette et al. obtained a DL of 12.8% for their monoclonal antibody-loaded PLGA microparticles when the s/o/w technique was used (Marquette et al., 2014b).

Therefore, the spray-drying of a water-in-oil (w/o) emulsion seems to be a suitable alternative for the production of protein-loaded microparticles. Indeed, spray-drying is a process that is reproducible and easily scalable. Moreover, compared to double emulsions techniques, the spray-drying of a w/o emulsion avoids the presence of an external aqueous phase, which may lead to the production of microparticles with higher DL (Giunchedi et al., 2001). However, the use of spray-drying for producing protein-loaded PLGA microparticles can show some drawbacks such as agglomeration issues and the low yields that are obtained due to the adhesion of the particles to the inner walls of the spray-drying apparatus (Makadia and Siegel, 2011).

The aim of this work is the production and the characterization of sustained-release formulations with high antibody-loaded levels by spray-drying a w/o emulsion. Polyclonal immunoglobulin G (IgG) was used as a model of antibody to evaluate the influence of different PLGA and of PLGA blends on the physicochemical properties of the produced microparticles.

2. Materials and methods

2.1. Materials

Polyclonal bovine IgG was used as a model of antibody (Equitech-Bio Inc., United States). PLGA 50:50 lactic acid: glycolic acid Resomer® RG502 (inherent viscosity 0.16–0.24 dL/g), RG503 (inherent viscosity 0.32–0.44 dL/g), RG505 (inherent viscosity 0.66–0.74 dL/g) and PLGA 75:25 lactic acid: glycolic acid Resomer® RG755S (inherent viscosity 0.50–0.70 dL/g) were purchased from Evonik Industries AG (Germany). Both poloxamer 188 (Lutrol® LF68) and poloxamer 407 (Lutrol® F127) were a gift from BASF (Germany). Polysorbate 80 was purchased from Ludeco (Belgium). D-(+)-trehalose dihydrate 99% was purchased from Alfa Aesar (Germany). Sodium hydroxide was obtained from VWR (Belgium). Dichloromethane (DCM), L-histidine, ethyl acetate (EtAc), di-sodium hydrogen phosphate and sodium dihydrogen phosphate monohydrate were purchased from Merck (Germany).

2.2. Methods

2.2.1. Production of IgG-loaded PLGA microparticles

Encapsulation of IgG into PLGA microparticles was performed using a two-step process.

In order to produce the w/o emulsion, PLGA polymers or blends of PLGA polymers (2.5% w/v) were dissolved in 20 mL of EtAc (organic phase) while IgG was solubilized in 2 mL of an aqueous buffered solution (pH 6.0) composed of L-histidine (0.3% w/v), trehalose (2.1% w/v) and poloxamer 407 (0.5% w/v). The w/o emulsion was obtained by high speed homogenization using a T25 Ultra-Turrax® high speed homogenizer (IKA, Germany) equipped with a S25N – 8G dispersing tool (IKA, Germany) set at 13,500 rpm for 1 min in an ice bath.

The w/o emulsion was spray-dried using a Mini Spray-Dryer B-290® (Büchi, Switzerland) equipped with a two-fluid nozzle (diameter: 0.7 mm). The inlet temperature was set at 50 °C; the drying air rate was set at 35 m^3/h; the gas spray flow was fixed at 800 L/h and the feed rate was set at 3 mL/min. The emulsion was kept under magnetic stirring into an ice bath during the entire spray-drying process. Outlet temperatures of 32–34 °C were observed.

The dried particles were collected and washed with 100 mL of a 0.1% w/v poloxamer 188 solution, followed by 100 mL of deionized water, to remove non encapsulated IgG. The particles were recovered on 0.22 µm polyvinylidene fluoride filters (Millipore, Ireland) and dried under vacuum for 48 h. The theoretical IgG loading was set at 23.0 ± 0.2% (w/w) for all the formulations.

2.2.2. Characterization of IgG-loaded PLGA microparticles

2.2.2.1. IgG loading and encapsulation efficiency. The amount of encapsulated IgG into the PLGA microparticles was determined by a bicinchoninic acid (BCA) protein assay. Briefly, 20 mg of microparticles were dissolved in 5 mL of a 0.1 M sodium hydroxide aqueous solution at room temperature. The samples were filtered on a 0.45 µm polyvinylidene fluoride filter (Pall, France). The Pierce® Microplate procedure was used to evaluate the amount of encapsulated IgG. DL and encapsulation efficiency (EE) were determined as follow:

$$IgG\ loading\ (\%) = \frac{amount\ of\ encapsulated\ IgG}{amount\ of\ microparticles} * 100$$

Equation 1: IgG loading (%)

$$EE\ (\%) = \frac{amount\ of\ encapsulated\ IgG}{initial\ amount\ of\ IgG} * 100$$

Equation 2: Encapsulation efficiency (%)

2.2.2.2. IgG stability evaluation after encapsulation. In order to evaluate the stability of IgG after encapsulation, 10 mg of the IgG-loaded microparticles were placed into a Nanosep® MF centrifugal device with a porosity of 0.2 µm (Pall, Mexico) containing 500 µL of DCM. The centrifugal device was put under stirring (600 rpm) during one hour at room temperature to solubilize the PLGA, using a Thermomixer comfort® tubes mixer (Eppendorf AG, Germany). Then, the organic

phase was removed by centrifugation at 12,000 rpm for 10 min and replaced by the same volume of fresh DCM. The sample was put under stirring (600 rpm) during 5 min and the resulting organic phase was then removed as previously described. The whole process was repeated twice. The precipitate was dried under vacuum for one hour and solubilized in 500 µL of a 200 mM pH 7.0 phosphate-buffered solution (PBS). Determination of the IgG concentration was performed by UV spectrophotometry at 280 nm using a SpectraMax M5 microplate reader (Molecular Devices, United States). Determination of the IgG monomer content was performed by size exclusion high-performance liquid chromatography (SE-HPLC) using a Hewlett Packard Agilent 1100 system equipped with a UV detector (Agilent Technologies, Germany). Separation was achieved with a TSKgel G3000SWXL 7.8 mm × 30.0 cm column (Tosoh Bioscience, Germany). The mobile phase was a 200 mM, pH 7.0 PBS. The flow rate and the volume of injection were set at 0.5 mL/min and 20 µL, respectively (Marquette et al., 2014a). These results were compared to a reference which consisted of raw IgG solubilized into 200 mM of PBS pH 7.0.

Extraction efficiency (ExE) was also determined, as follow:

$$ExE\ (\%) = \frac{amount\ of\ extracted\ IgG}{amount\ of\ encapsulated\ IgG\ (determined\ by\ BCA)} * 100$$

Equation 3: Extraction efficiency (%)

2.2.2.3. Particle size distribution and surface morphology. The particle size distribution of the produced microparticles was determined in water with a Mastersizer® 3000 Hydro MV (Malvern, United Kingdom), using refractive indexes of 1.33 and 1.55 for water and PLGA, respectively. The particles were suspended into a 0.5% w/v polysorbate 80 aqueous solution and each sample was sonicated for 180 s (by 30-second increments) prior to measurement. The particle size distribution was characterized by the volume median diameter d (0.5), the volume mean diameter D[4;3] and the d(0.9).

The morphology of the produced microparticles was evaluated using a Su-70 Hitachi scanning electron microscope (Hitachi, Germany). Before the analysis, the samples were placed on an adhesive carbon tap and were coated with 15–20 nm of gold at 40 mA under argon atmosphere for 90 s at 6×10^{-2} mbar. During the analysis, the samples were subjected to a 10 keV acceleration voltage.

2.2.2.4. Differential scanning calorimetry (DSC). The glass transition temperature (T_g) of PLGA microparticles was determined by DSC, using a DSC Q2000 equipment (TA Instruments, Belgium) with Tzero aluminium hermetic pans sealed with aluminium lids. The samples were analyzed using a heat/cool/heat mode to erase their thermal history. They were heated from −20 °C to 80 °C, then cooled down to −20 °C before being heated up to 150 °C using a cooling/heating rate set at 10 °C/min. The experiments were run under a nitrogen flow of 50 mL/min. The T_g of the polymers corresponded to the inflection point observed during the second heating cycle.

2.2.2.5. In vitro release studies. In order to evaluate the release profiles of IgG from PLGA microparticles, 40 mg of microparticles were placed in 1 mL of a 200 mM pH 7.0 PBS into 2 mL tubes and incubated at 37 °C under continuous stirring (600 rpm) using a Thermomixer comfort® tubes mixer (Eppendorf AG, Germany). At predetermined time intervals, the samples were centrifuged during 15 min at 5260 rpm. The supernatant (1 mL) was collected and filtrated on a 0.45 µm polyvinylidene fluoride filter (Pall, France). The microparticles were suspended again in 1 mL of fresh PBS for further testing. The filtrated supernatant was analyzed by UV spectrophotometry at 280 nm using a SpectraMax M5 microplate reader (Molecular Devices, United States) to determine IgG concentration.

2.2.2.6. Determination of release parameters by nonlinear regression. Cumulative IgG release data were fitted by nonlinear curve fitting with Excel, using Equation 4, developed by Duvvuri et al. (Duvvuri et al., 2005),

$$F = A[1 - -\exp(-K_1 * T)] + B/(1 + \exp[-K_2 * (T - -T_{50})])$$

Equation 4: Calculation of the release of IgG

F being the fraction of IgG released; A (that can also be described as the burst release) and B being the percentages of encapsulated IgG released during phase I and phase III, respectively; K_1 and K_2 being the release rate constants during phase I and phase III, respectively; and T_{50} being the time taken to release 50% of the encapsulated IgG. The correlation coefficient was determined to evaluate the fitting of the model with experimental data.

2.2.3. Data analysis

All experiments were performed in triplicate, unless otherwise specified. The results are expressed as a mean ± standard deviation. Statistical significance was determined at $p < 0.05$ using ANOVA and Student's *t*-tests.

3. Results

3.1. Particle size and surface morphology

All formulations showed a d(0.5), a D[4,3] and a d(0.9) lower than 10 µm, 15 µm and 35 µm, respectively, except for F1 (Resomer® RG502) which presented larger diameters (436.0 ± 74.5 µm, 391.0 ± 40.8 µm and 835.0 ± 24.1 µm for the d(0.5), the D[4,3] and the d(0.9), respectively) (Table 1). The lowest values (3.9 ± 0.0 µm, 5.4 ± 0.0 µm and 10.8 ± 0.1 µm for the d(0.5), the D[4,3] and the d (0.9), respectively) were obtained with the use of Resomer® RG755S. A significant decrease in the D[4,3] values of the microparticles was observed when the inherent viscosity of the polymer was increased (391.0 ± 40.8 µm, 13.2 ± 0.1 µm and 9.2 ± 0.1 µm for the D[4,3] of F1, F2 and F3, respectively) (Student's *t*-test, $p < 0.05$). Similar trends

Table 1
Particle size distribution characterized by d(0.5), D[4,3] and d(0.9) of individualized particles, measured with a Mastersizer® 3000 Hydro MV laser diffractometer and glass transition temperatures (Tg) of the formulations determined by DSC analysis.

Formulation ID (polymer(s)-ratio)	Inherent viscosity (dL/g)	Lactic acid: glycolic acid ratio	d(0.5) (µm)	$D_{[4;3]}$ (µm)	d(0.9) (µm)	T_g (°C)
F1 (Resomer® RG502)	0.16–0.24	50:50	436.0 ± 74.5	391.0 ± 40.8	835.0 ± 24.1	36.8 ± 0.4
F2 (Resomer® RG503)	0.32–0.44	50:50	9.0 ± 0.0	13.2 ± 0.1	30.6 ± 0.2	40.3 ± 0.3
F3 (Resomer® RG505)	0.66–0.74	50:50	6.6 ± 0.0	9.2 ± 0.1	20.3 ± 0.2	41.3 ± 0.4
F4 (Resomer® RG755S)	0.50–0.70	75:25	3.9 ± 0.0	5.4 ± 0.0	10.8 ± 0.1	45.9 ± 2.2
F5 (1:1 Resomer® RG502/RG505)	–	–	7.8 ± 0.0	10.8 ± 0.1	23.9 ± 0.1	39.0 ± 0.3
F6 (1:1 Resomer® RG503/RG505)	–	–	5.9 ± 0.0	7.9 ± 0.0	16.7 ± 0.1	41.4 ± 0.3
F7 (4:1 Resomer® RG503/RG505)	–	–	7.1 ± 0.0	11.1 ± 0.2	23.0 ± 0.2	41.2 ± 0.5
F8 (2:1 Resomer® RG503/RG505)	–	–	7.4 ± 0.0	12.0 ± 0.3	25.6 ± 0.5	41.2 ± 0.1
F9 (1:2 Resomer® RG503/RG505)	–	–	7.1 ± 0.3	9.7 ± 0.1	22.7 ± 3.0	43.2 ± 2.2
F10 (1:4 Resomer® RG503/RG505)	–	–	5.2 ± 0.0	7.2 ± 0.0	16.0 ± 0.1	41.8 ± 0.5

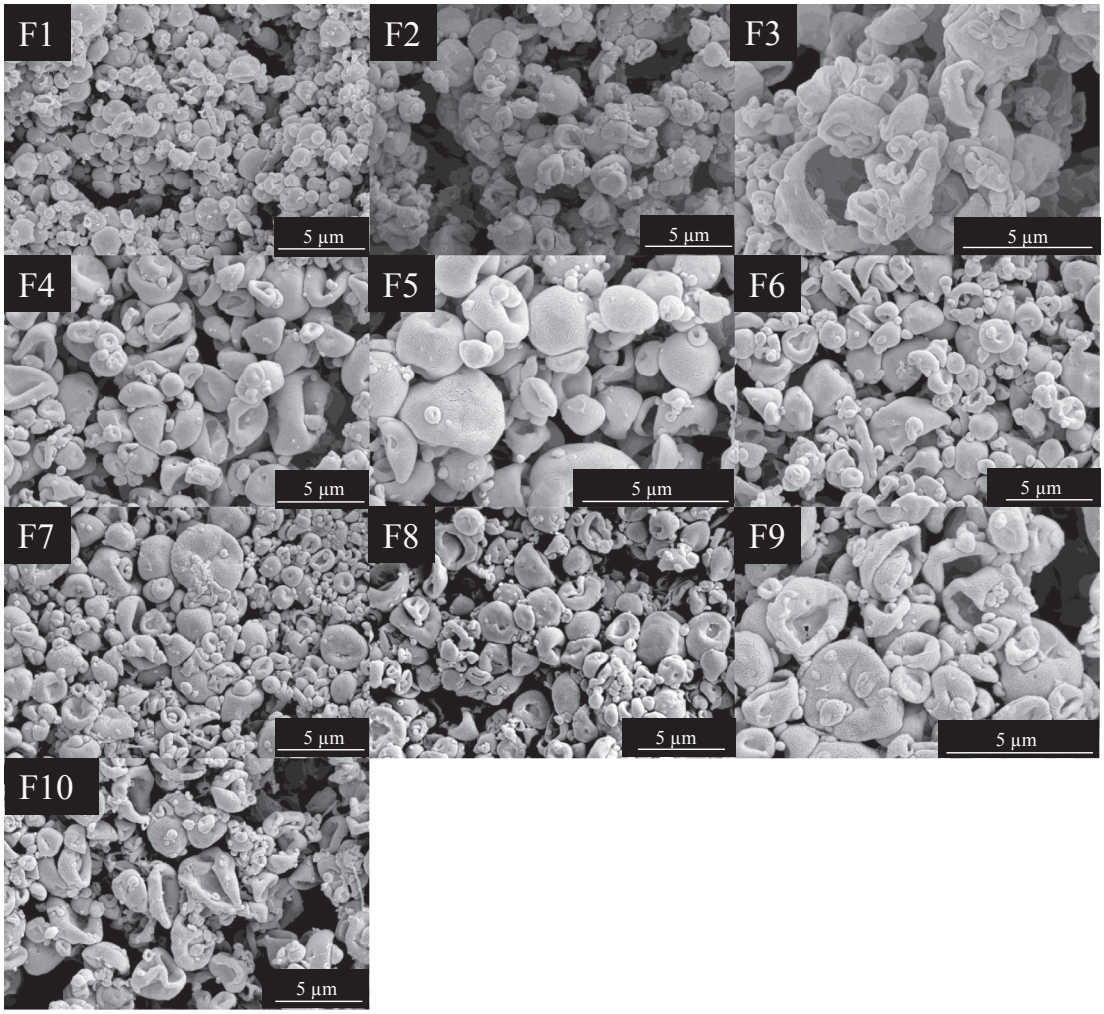

Fig. 1. SEM pictures of IgG-loaded PLGA microparticles. F1, F2, F4, F6, F7, F8 and F10: magnification x6000. F3: magnification x9000. F5 and F9: magnification x10000.

could be observed with the increase of the lactide content (D[4,3] decrease from 9.2 ± 0.1 µm with F3 to 5.4 ± 0.0 µm with F4). The d (0.5), D[4,3] and d(0.9) of the formulations produced with PLGA blends were between the d(0.5), D[4,3] and d(0.9) values of the microparticles obtained with the use of the single PLGA except for F6 (1:1 Resomer® RG503/RG505) and F10 (1:4 Resomer® RG503/RG505).

The morphologies of the particles are shown in Fig. 1. For all formulations, non-porous particles with a doughnut-liked shape were obtained. Besides, different populations in terms of particle size could be observed.

3.2. Differential scanning calorimetry

The T_g of the formulations ranged from 36.8 ± 0.4 °C to 45.9 ± 2.2 °C, the lowest and highest values being obtained with the use of Resomer® RG502 and Resomer® RG755S, respectively (Table 1). A significant increase of T_g was observed with the increase of the inherent viscosity of the polymer (from 36.8 ± 0.4 °C for F1 to 41.3 ± 0.4 °C for F3) (Student's *t*-test, $p < 0.05$) while no significant difference was observed with the increase of lactide content (Student's *t*-test, $p > 0.05$). For all the formulations produced from polymer blends, a single T_g was observed. Adding Resomer® RG505 to the matrix made of Resomer® RG502 allowed a significant increase of the T_g from 36.8 ± 0.4 °C to 39.0 ± 0.3 °C (Student's *t*-test, $p < 0.05$) while T_g values were not significantly modified when Resomer® RG505 was blended with Resomer® RG503 (ANOVA, $p > 0.05$).

3.3. IgG loading and encapsulation efficiency

All formulations, except F1, showed IgG loadings and EE above 16.0% and 70%, respectively (Table 2). Indeed, F1 (Resomer® RG502) presented lower values (9.9 ± 0.4% and 42.9 ± 1.8% for the IgG loading and the EE, respectively). Increasing the inherent viscosity of the polymer allowed a significant increase of IgG loading and EE (from 9.9 ± 0.4% and 42.9 ± 1.8% for the IgG loading and the EE for F1, respectively to 20.1 ± 0.7% and 87.4 ± 3.0% for F3) (Student's *t*-test, $p < 0.05$). A significant decrease of IgG loading and EE was obtained with the increase of the lactide ratio of the polymer (16.8 ± 0.4% of IgG loading and 73.1 ± 1.8% of EE for F4 in comparison to 20.1 ± 0.7% of IgG loading and 87.4 ± 3.0% of EE for F3) (Student's *t*-test, $p < 0.05$). Adding Resomer® RG505 to the polymeric matrix led to a significant increase of IgG and EE (18.4 ± 0.1% and 85.4 ± 1.5% for IgG loading and EE, respectively) in comparison to the use of Resomer® RG502 alone (9.9 ± 0.4% and 42.9 ± 1.8% for IgG loading and EE, respectively) (Student's *t*-test, $p < 0.05$). For the formulations produced from the blend of Resomer® RG503 and RG505, IgG loadings and EE were significantly higher than those obtained with the use of Resomer® RG503 alone, whatever the ratio was (Student's *t*-test, $p < 0.05$). In particular, there was no significant difference between IgG loadings and EE obtained for Resomer RG505 and for the blends of Resomer® RG503 and RG505, whatever the ratio was (ANOVA, $p > 0.05$).

Table 2
IgG loading and Encapsulation Efficiency obtained by BCA protein assay; Extraction Efficiency and monomer content obtained for the formulations by microparticles dissolution followed by IgG solubilization.

Formulation ID (polymer(s)-ratio)	Inherent viscosity (dL/g)	Lactic acid: glycolic acid ratio	IgG loading (% w/w)	EE (%)	ExE (%)	Monomer content (%)
F1 (Resomer® RG502)	0.16–0.24	50:50	–	–	–	58.2 ± 0.6
F2 (Resomer® RG503)	0.32–0.44	50:50	9.9 ± 0.4	42.9 ± 1.8	66.0 ± 2.5	57.2 ± 0.7
F3 (Resomer® RG505)	0.66–0.74	50:50	18.5 ± 0.3	80.6 ± 1.2	85.8 ± 1.7	64.5 ± 0.6
F4 (Resomer® RG755S)	0.50–0.70	75:25	20.1 ± 0.7	87.4 ± 3.0	89.3 ± 1.6	63.5 ± 0.5
F5 (1:1 Resomer® RG502/RG505)	–	–	16.8 ± 0.4	73.1 ± 1.8	88.2 ± 9.8	61.9 ± 1.0
F6 (1:1 Resomer® RG503/RG505)	–	–	18.4 ± 0.1	79.9 ± 0.6	93.5 ± 3.8	61.8 ± 0.2
F7 (4:1 Resomer® RG503/RG505)	–	–	19.6 ± 0.4	85.4 ± 1.5	90.5 ± 1.3	61.5 ± 0.6
F8 (2:1 Resomer® RG503/RG505)	–	–	19.6 ± 0.6	85.3 ± 2.7	89.5 ± 2.6	61.4 ± 0.3
F9 (1:2 Resomer® RG503/RG505)	–	–	19.5 ± 0.4	84.7 ± 1.6	91.4 ± 1.8	62.8 ± 0.3
F10 (1:4 Resomer® RG503/RG505)	–	–	20.3 ± 0.2	88.5 ± 1.0	89.9 ± 5.5	63.4 ± 0.4

3.4. IgG stability after encapsulation

For all formulations, more than 80% of encapsulated IgG have been extracted from the microparticles except for F1 (Resomer® RG502) for which the percentage of extracted IgG was sensibly lower (ExE value of 66.0 ± 2.5%) (Table 2). Adding Resomer® RG505 to the polymeric matrix led to a significantly higher percentage of IgG extracted (ExE value of 93.5 ± 3.8%) in comparison to the use of Resomer® RG502 alone (66.0 ± 2.5%) (Student's *t*-test, $p < 0.05$). Meanwhile, for the formulations produced from the blend of Resomer® RG503 and RG505, no significant difference with the use of Resomer® RG503 alone was observed, regardless of the ratio (ANOVA, $p > 0.05$).

A significantly higher monomer content than that obtained from the reference was observed for all formulations (Student's *t*-test, $p < 0.05$), except for F1 (Resomer® RG502) for which the monomer content was not significantly different (Student's *t*-test, $p > 0.05$) (Table 2). Adding Resomer® RG505 to the polymeric matrix allowed a significant increase of the monomer content (61.8 ± 0.2%) compared to the use of single Resomer® RG502 (57.2 ± 0.7%) (Student's *t*-test, $p < 0.05$). For the formulations produced from the blend of Resomer® RG503 and RG505, all monomer content values were lower than those obtained with the use of Resomer® RG503 alone.

3.5. In vitro release profiles

Triphasic release profiles were observed for all formulations (Figs. 2–4). Indeed, they were all characterized by (i) an initial burst release (phase I), (ii) a lag phase (phase II) and (iii) a second release (phase III). Within 29 weeks, encapsulated IgG was completely released from the microparticles, except for both formulations F1 (Resomer® RG502) and F4 (Resomer® RG755S) as the maximal percentage of release only reached only 82.7 ± 2.9% and 77.0 ± 0.2%, respectively.

Cumulative IgG release data were fitted by non-linear regression analysis to Equation 4. Correlation coefficients between 0.994 and 0.999 were obtained. Regarding the use of single PLGA 50:50 lactic acid:glycolic acid, it could be seen that K_1 significantly decreased (Student's *t*-test, $p < 0.05$) with the increase of the inherent viscosity (from 56.3 ± 11.4/day for F1 to 37.6 ± 1.7/day for F3), underlining an increase in phase I duration (Table 3). Moreover, the highest burst release (represented by the A value) was obtained with the use of Resomer® RG503, while formulations produced from both Resomer® RG502 and RG505 provided similar values. Increasing the inherent viscosity of the polymer led to significant increases of lag phase duration (T_{50} values from 58 ± 6 days for F1 to 145 ± 11 days for F3) and of the percentages of IgG released during phase III (B values from 41.1 ± 3.6% for F1 to 75.5% ± 0.9% for F3) (Student's *t*-test, $p < 0.05$). A significantly lower drug release constant during phase III was obtained when Resomer® RG505 was used (Student's *t*-test, $p < 0.05$). Using a PLGA with a higher lactide ratio led to a significantly higher burst release (A values of 40.1 ± 1.8% and 54.3 ± 1.5% for F3 and F4, respectively) (Student's *t*-test, $p < 0.05$)

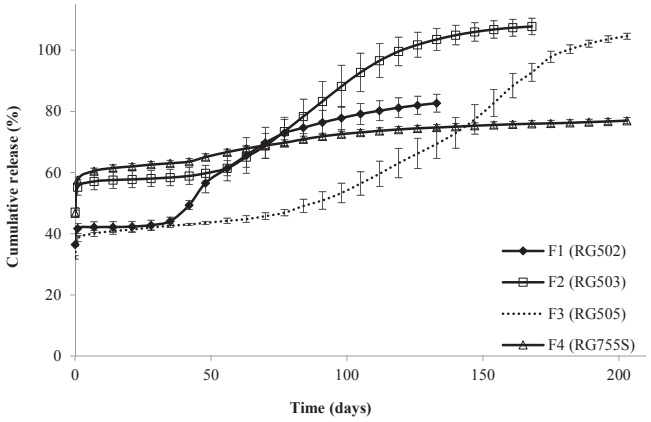

Fig. 2. *In vitro* IgG release profiles of formulations produced by using individual polymers (mean ± SD; n = 3).

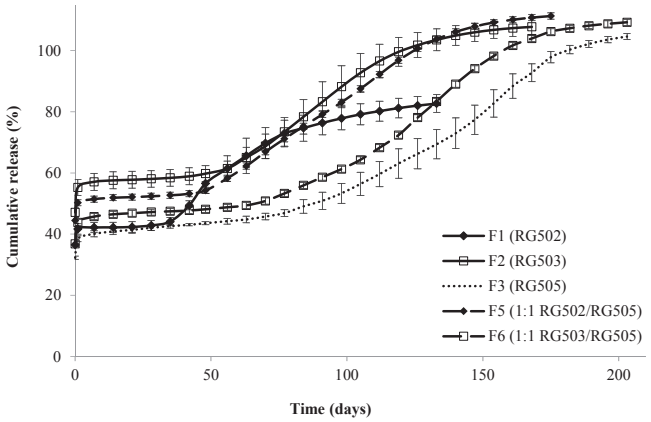

Fig. 3. *In vitro* IgG release profiles of formulations produced by using either Resomer® RG502 or RG503 with Resomer® RG505 in a 1:1 blend. For comparison, formulations of the corresponding individual polymers were also added into the figure (mean ± SD; n = 3).

and meanwhile a lower percentage of IgG released during phase III (B values from 75.5 ± 0.9% for F3 to 22.5 ± 1.4% for F4) . It also allowed a significant decrease of lag phase duration (T_{50} values from 145 ± 11 days for F3 to 49 ± 5 days for F4) and of drug release constant during phase III (K_2 values from 0.034 ± 0.002/day for F3 to 0.028 ± 0.002/day for F4) (Student's *t*-test, $p < 0.05$).

Using a polymeric blend composed of Resomer® RG502 and RG505 allowed a modulation of release characteristics (Table 3). Thus, in comparison to the use of single Resomer® RG502, a significant increase of burst release and of IgG released during phase III was obtained as shown by the increase of A and B values (Student's *t*-test, $p < 0.05$). Besides, a significant decrease of drug release constant during phase III

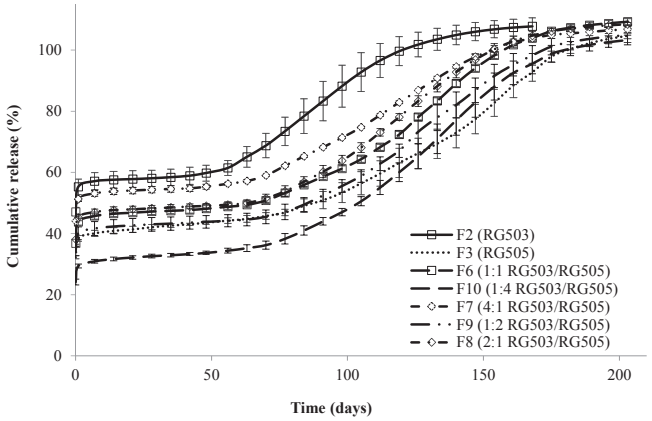

Fig. 4. *In vitro* IgG release profiles of formulations produced by using Resomer® RG503/RG505 blends at different ratios. For comparison, formulations of the corresponding individual polymers were also added into the figure (mean ± SD; n = 3).

was observed (Student's *t*-test, $p < 0.05$). Meanwhile, a significantly lower T_{50} value (Student's *t*-test, $p < 0.05$), underlining a shorter lag phase than the one obtained with the use of Resomer® RG505 alone could be seen.

Regarding the use of Resomer® RG503/RG505 blends, an increase of burst release (A value) was observed when the amount of Resomer® RG505 was decreased (Table 3). Meanwhile, a decrease of the percentage of IgG released during phase III, as shown by the decrease of the B value, was obtained. Decreasing the amount of Resomer® RG505 also led to shorter lag phases as underlined by the T_{50} values. K_2 values of Resomer® RG503/RG505 blends were significantly different from the values obtained for both Resomer® RG503 and Resomer® RG505 used alone (Student's *t*-test, $p < 0.05$).

4. Discussion

Nowadays, protein encapsulation into PLGA microparticles is usually performed using double emulsion techniques such as w/o/w or s/o/w emulsions, followed by a solvent evaporation and/or extraction step. However, a significant decrease of the DL is commonly observed due to the leakage of the protein into the external aqueous phase. For this reason, alternative formulation strategies have emerged. In this work, the spray-drying of a w/o emulsion was selected as it offers considerable advantages compared to the current encapsulation techniques such as its easiness of use and of scaling-up. The possibility of producing, by spray-drying a w/o emulsion, PLGA microparticles with high antibody-loaded levels with appropriate physicochemical properties has been studied. Polyclonal IgG was used as a model of antibody and multiple PLGA were also evaluated to optimize IgG stability, DL and dissolution profiles.

4.1. Influence of the PLGA

Regardless of the formulations, microparticles with a doughnut-liked shape and a smooth surface were obtained. This could be attributed to the deformation of the produced particles during a too long solvent evaporation phase since the solvents used are ethyl acetate and water, which is not the case when dichloromethane is used for example. Indeed, Wang and co-workers have already mentioned such observation during the production of etadinazole-loaded microparticles by spray-drying using ethyl acetate (Wang and Wang, 2002).

As it can be seen in Table 2, three of the single PLGA (Resomer® RG503, RG505 and RG755S) tested led to IgG loadings and EE above 16.0% and 70%, respectively. An increase of the EE was observed when the inherent viscosity of the PLGA increased. It could be explained by a reduced diffusion of the protein to the surface of the microparticles when the viscosity of the solution of polymer was increased (Gaignaux et al., 2012). Meanwhile, a decrease of the IgG loading as well as of the EE was obtained when the lactide content was increased. Due its higher lactide content, Resomer® RG755S is more hydrophobic than Resomer® RG505. Thus, it could be hypothesized that IgG might be characterized by a lower affinity for Resomer® RG755S than for Resomer® RG505 and it could migrate more easily to the solvent/air interface.

In addition to showing significantly lower IgG loading and EE, microparticles made of Resomer® RG502 were also unsuitable in terms of particle size. Indeed, they were characterized by a mean diameter of 391 µm which is not suitable for injection (Table 1). It could be explained by the agglomeration of individualized particles as observed by SEM analysis (Fig. 1). The T_g value of these microparticles (37 °C) was close to the outlet temperature observed during the spray-drying process (32–34 °C), which could have led to agglomeration issues. However, the use of Resomer® RG503, RG505 or RG755S allowed obtaining particles characterized by a mean diameter lower than 125 µm. The decrease in the mean diameter obtained when increasing the inherent viscosity of PLGA underlined that polymer viscosity was not the determining factor for particle size during the spray-drying process (Wang and Wang, 2003).

In order to evaluate IgG stability after the encapsulation process, the antibody was extracted from the produced microparticles. There was an increase of ExE with the increase of inherent viscosity (Table 2). Indeed, approximately 35% of IgG was not recovered from the formulation based on the use of Resomer® RG502 alone. This percentage of non-recovery decreased with the increase of inherent viscosity, to approximately 10% when Resomer® RG505 was used. Incomplete recovery of IgG could be explained by a degradation of IgG during the encapsulation process due to the presence of interfaces or contact with PLGA (Van de Weert et al., 2000). Such degradation could prevent its extraction from the polymeric microparticles. Thus, using a PLGA with a higher inherent viscosity allowed a better protection against IgG degradation. It could be explained by the increased stability of the emulsion with the increase of viscosity of the dispersing phase. Besides, the lowest monomer content was obtained with the use of Resomer® RG502,

Table 3
Release parameters obtained by non-linear regression analysis fit of IgG release data to Equation 4.

Formulation ID (polymer(s)-ratio)	A (%)	K_1 (/day)	T_{50} (days)	B (%)	K_2 (/day)
F1 (Resomer® RG502)	40.1 ± 1.8	56.3 ± 11.4	58 ± 6	41.1 ± 3.6	0.075 ± 0.012
F2 (Resomer® RG503)	56.3 ± 2.6	42.9 ± 2.2	90 ± 7	51.7 ± 0.6	0.060 ± 0.008
F3 (Resomer® RG505)	40.1 ± 1.8	37.6 ± 1.7	145 ± 11	75.5 ± 0.9	0.034 ± 0.002
F4 (Resomer® RG755S)	54.3 ± 1.5	35.8 ± 0.6	49 ± 5	22.5 ± 1.4	0.028 ± 0.002
F5 (1:1 Resomer® RG502/RG505)	49.0 ± 1.8	52.0 ± 1.0	94 ± 1	64.4 ± 0.9	0.044 ± 0.004
F6 (1:1 Resomer® RG503/RG505)	45.9 ± 0.1	38.4 ± 2.4	126 ± 1	66.4 ± 1.8	0.044 ± 0.001
F7 (4:1 Resomer® RG503/RG505)	52.4 ± 1.2	43.1 ± 1.2	114 ± 2	56.0 ± 0.7	0.043 ± 0.002
F8 (2:1 Resomer® RG503/RG505)	46.8 ± 1.0	40.0 ± 4.2	119 ± 1	63.0 ± 0.5	0.048 ± 0.001
F9 (1:2 Resomer® RG503/RG505)	41.6 ± 2.4	41.0 ± 1.7	129 ± 3	66.3 ± 2.4	0.045 ± 0.001
F10 (1:4 Resomer® RG503/RG505)	31.2 ± 0.7	34.8 ± 2.7	130 ± 5	76.3 ± 0.3	0.043 ± 0.003

confirming that this PLGA was detrimental to IgG stability. Meanwhile, using Resomer® RG503, RG505 or RG755S allowed maintaining IgG stability through the encapsulation process.

The release profiles of IgG from the PLGA microparticles were greatly influenced by the type of PLGA that was used (Figs. 2–4). Correlation coefficients values ranged from 0.994 to 0.999, assessing the validity of using Equation 4 to describe IgG release profiles (Table 3). Phase I corresponds to the initial diffusion step. Both A and K_1 represent the percentage of encapsulated IgG that was released and the release rate constant during this phase, respectively. Phase II corresponds to the lag phase in which a little amount or no drug was released. T_{50} allows an evaluation of the duration of phase II. Phase III is the second release phase in which the drug diffuses from the microparticles. There has been recent evidence that the onset of phase III might be attributed to microparticles swelling, which occurs as soon as the molecular weight of PLGA has decreased to a critical value (Gasmi et al., 2015). Both B and K_2 represent the percentage of encapsulated IgG that was released and the release constant during this phase, respectively. The increase of the inherent viscosity leading to a decrease of K_1 could be explained by a slower diffusion of IgG due to the higher molecular weight of the polymer. An increase of the burst release was observed with a higher lactide content. It could be explained by an enhanced migration of IgG to the outer surface of the microparticles due to its lower affinity for a more hydrophobic PLGA. The duration of the lag phase (phase II) increased with the increase of the inherent viscosity. It has been observed that during the release tests, the molecular weight of PLGA needed to decrease to a critical value of approximately 10,000 Dalton to initiate the third phase (Bodmer and Traechslin, 1992). Considering that the inherent viscosity increases with the molecular weight, the highest the inherent viscosity is, the longest it takes for the PLGA to reach this critical molecular weight value and the longest the lag phase duration will be. Percentages of IgG released during phase III increased with the increase of inherent viscosity, underlining better retention of IgG into the polymeric matrix with the increase of inherent viscosity. Incomplete release observed from the formulations produced with the use of Resomer® RG502 and RG755S could be due to IgG degradation during the release study (Giteau et al., 2008).

Thus, Resomer® RG505, characterized by an inherent viscosity of 0.66–0.74 dL/g and a 50:50 lactic acid: glycolic acid ratio, was the most interesting among the PLGA tested since it allowed: (i) obtaining DL and EE of approximately 20% and 87%, respectively, (ii) maintaining IgG stability during the encapsulation process and (iii) obtaining a release profile with a limited burst release of 40% and a release phase (phase III) duration of approximately 3 months. However, the lag phase duration (approximately 3 months) was the limitation of the use of this single polymer.

4.2. Optimization of the formulations using PLGA blends

Polymer blending has been successfully applied to reduce the lag phase duration of encapsulated drugs such as dexamethasone, ganciclovir and LHRH superagonist analogue leuprolide acetate which were prepared by emulsification followed by solvent extraction/evaporation techniques (Duvvuri et al., 2006; Ravivarapu et al., 2000; Wang et al., 2014). Therefore, the interest of using mixtures of PLGA for the encapsulation of our model of antibody by spray-drying a w/o emulsion was evaluated in this study.

Obtaining appropriate characteristics in terms of IgG stability and loading, EE and meanwhile release profile was not possible with the use of a single PLGA. Thus, it was decided to blend Resomer® RG505 (inherent viscosity 0.66–0.74 dL/g) which showed the most interesting results except for the lag phase with a PLGA having a lower inherent viscosity since it could allow a decrease of the lag phase duration. Resomer® RG502 (inherent viscosity 0.16–0.24 dL/g) and RG503 (inherent viscosity 0.32–0.44 dL/g) were tested in combination with Resomer® RG505 at a single weight ratio for Resomer® RG502 while a study of the influence of the weight ratio of PLGA used into the blend on microparticles characteristics was performed for Resomer® RG503.

For all blends, a single T_g was observed, underlining the miscibility of the polymers at the ratios studied (Duvvuri et al., 2006). Blending Resomer® RG505 with Resomer® RG502 significantly improved microparticles characteristics in comparison to the use of Resomer® RG502 alone. In particular, even with 50% w/w of Resomer® RG502 into the blend, particles with a mean diameter lower than 125 µm and EE as high as 80% were obtained. Besides, IgG stability could be maintained during the encapsulation process (Table 2) and a complete release of IgG during the dissolution study was observed (Fig. 3). Meanwhile, a significant decrease of lag phase duration of approximately one month was possible in comparison to the use of Resomer® RG505 alone. Faster polymer degradation and microparticles erosion due to the presence of a polymer with a lower inherent viscosity could explain this observation (Wang et al., 2014).

Regarding Resomer® RG503/RG505 blends, a significant improvement of IgG loading and EE was observed in comparison to the use of Resomer® RG503 alone (Table 2). More specifically, similar IgG loadings and EE were obtained with the use of Resomer® RG505 alone and with the blends, even with the lowest percentage of Resomer® RG505 into the formulation. This indicates that IgG loading and EE were mainly governed by the presence of Resomer® RG505. However, no significant difference regarding IgG loading and EE was observed between the different Resomer® RG503/RG505 mixtures, indicating that modifying the ratio of the PLGA into the mixture did not affect IgG loading and EE, contrary to what was observed for the encapsulation of a small molecule such as pentamidine (Graves et al., 2004). The influence of the Resomer® RG503/RG505 ratio used on release characteristics was clearly underlined (Fig. 4). Thus, by increasing the percentage of the PLGA with a low inherent viscosity into the blend, IgG could migrate more easily onto the surface of the microparticles, leading to higher burst releases and consequently lower IgG released after the erosion of the microparticles. It also allowed a decrease of the lag phase duration as already mentioned by Duvvuri et al. (Duvvuri et al., 2006). It was interesting to see that a minimum of 50% of Resomer® RG503 into the mixture was required to significantly reduce the lag phase duration in comparison to the use of single Resomer® RG505.

Optimization of the formulations by using polymer blending was investigated there. For all blends, particle size with a mean diameter lower than 125 µm and IgG loadings and EE above 18% and 75%, respectively were obtained. Besides, IgG stability was maintained during the encapsulation process. An influence of the polymers ratios on the overall IgG release was clearly underlined. Thus, a modulation of the lag phase duration could be effectively performed by varying the polymers ratios into the blend. In particular, the 1:1 w/w Resomer® RG503/RG505 ratio allowed to obtain a shortened lag phase while limiting the burst release. However, none of the *in vitro* release profiles presented here would allow a continuous IgG release.

5. Conclusion

The possibility of producing highly antibody-loaded PLGA microparticles by spray-drying a w/o emulsion was assessed. Among the PLGA that were tested, Resomer® RG505 was the most interesting since it allowed obtaining DL and EE of approximately 20% and 87%, respectively, maintaining IgG stability during the encapsulation process and obtaining a release profile with a limited burst release and a release phase duration of approximately 3 months. However, the lag phase duration of 3 months was limiting the use of this PLGA as a single component for the polymeric matrix. Polymer blending appeared as a successful strategy to decrease the lag phase duration. Indeed, the use of minimum 50% of a PLGA with a lower inherent viscosity such as Resomer® RG502 or RG503 into the blend significantly shortened the lag phase up to 25 days. Meanwhile, IgG loadings and EE above 18%

and 75% could be obtained and IgG stability was preserved with all blends thanks to the presence of Resomer® RG505. Nonetheless, it was not possible to achieve a continuous *in vitro* release of IgG during this study. The necessity of suppressing the lag phase by further optimizing the formulations will be evaluated by conducting pharmacokinetics studies, because faster release kinetics are usually observed *in vivo*. At that point, the spray-drying of a w/o emulsion appeared to be a promising formulation strategy to produce highly loaded antibody-loaded microparticles with sustained-release properties.

Declaration of Competing Interest

None.

Acknowledgements

The authors would like to thank UCB Pharma S.A. and the Walloon region for funding this project.

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